TAN-293 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

Hiroshi TOMIYAMA, et al.

Serial No.: Unassigned Group:

Filed: Concurrently Examiner:

FOR: NON-MUCIN TYPE SYNTHETIC COMPOUNDS OR ITS CARRIER CONJUGATED COMPOUNDS

Date: August 6, 2001

The Hon. Commissioner of
Patents and Trademarks
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

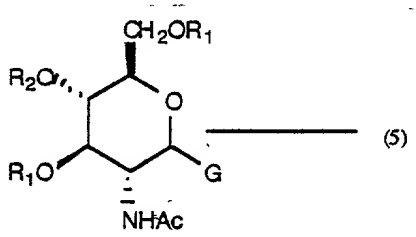
Preliminary to examination, please amend the herewith filed application as follows:

IN THE SPECIFICATION

Please amend the specification as follows:

In the paragraph on Page 7, at lines 14-18:

That is to say the process for the preparation of N-acetyl galactosamine derivatives, general formula (6) can be prepared from the [invention] inversion of OR_2 group to OR_1 group at C-4 position in N-acetyl glucosamine derivatives, general formula (5).



(5)

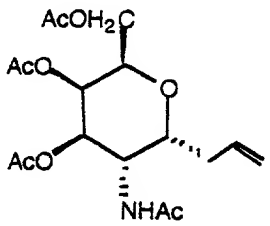
wherein [OR_1] $\underline{R_1}$ is H or a protecting group of a hydroxy group such as acetyl group; R_2 is a leaving group such as tosylate, trifluoromesylate or methansulfonate; G is allyl or protected hydroxyl groups.

In the paragraph at Page 39, lines 1-4:

Our results also shows the potent immunogenicity of metabolic and catabolic stable "C-glycopeptoid" with or even without carrier protein. On the other hand, Danishefsky's team reported the O-Tn, O-STn, O-TF antigens [dose not] have less potent immunogenicity themselves, but attached to carrier proteins such as KLH. (S.J. Danishefsky et al, 1998, 120, 1427-14285.)

In the paragraph at Page 45, lines 4-21:

The preparation of 3-(2-acetylamino-3,4,6-tetra-O-acetyl-2-deoxy-α-D-[gluctopyranosyl] <u>galactopyranosyl</u>)-1-propene (compound 1b-2)

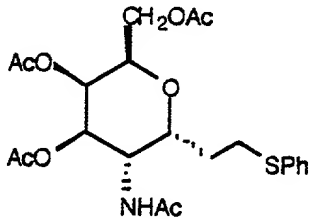


To N-acetylglucosamine 100g (0.45mol) was added acetyl chloride (200ml) at 0°C and stirred for 23h. After the reaction, the mixture was extracted with chloroform and the mixture was poured into ice cold water and stirred for 10 min. The organic layer was neutralized by satd. $NaHCO_3$ and dried (Na_2SO_4). The solvent was removed under reduced pressure. Diethyl ether was added to the residue and the resulting precipitate was collected. 117g

(71%) of 2-acetylamino-1-chloro-3,4,6-tetra-O-acetyl-2-deoxy-α-D-[gluctose] galactose was obtained as a colorless solid. To a solution of the obtained compound (78g, 0.21mol) in tetrahydrofuran (400ml) was added allyltributyltin (198ml, 0.64mol) and 2,2'-azobisisobutyronitrile (AIBN) (3.4g, 0.02mol). The reaction mixture was heated to 80°C and stirred for 16h under argon atmosphere. The reaction mixture was concentrated under reduced pressure. The resulting residue was purified by silicagel column chromatography (AcOEt: n-hexane=4:1). The mixture of allyl compound (1.62g) was obtained. To a solution of the obtained mixture in aceton (10ml) was added 1% HCl (6ml) and stirred for 2h. The mixture was concentrated under reduced pressure and the residue was extracted with chloroform (30ml). The organic layer was neutralized by satd. $NaHCO_3$ and dried (Na_2SO_4). The solvent was removed under reduced pressure. The resulting residue was purified by silicagel column chromatography AcOEt: n-hexane=4:1). 73g (92%) of the objective compound was obtained as a colorless solid.

In the paragraph at Page 52, lines 13-22:

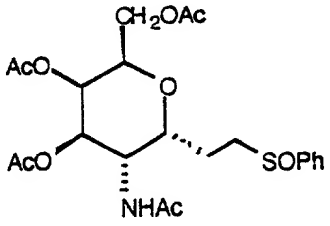
The preparation of 3-(2-acetylamino-[3,4-tri]3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-1-phenylthioethane (compound 4-1)



The compound (0.25g, 0.67mmol) obtained from the above mentioned Example 7 was dissolved in pyridine (3ml), tributylphosphine (0.42ml) and diphenyldisulfide (0.32g) were added to the solution. The mixture was stirred for 3h at 60°C under argon atmosphere. The reaction mixture was extracted with ethyl acetate and washed with water and brine. After drying ($MgSO_4$), the solvent was removed under reduced pressure. The resulting residue was purified by silicagel column chromatography (BW-200, AcOEt:n-Hexane=10:1). 0.18g (56%) of the objective thiophenyl compound was obtained as a colorless oil.

In the paragraph at Page 53, lines 5-13:

The preparation of 3-(2-acetylamino-[3,4-tri]3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-1-phenylsulufenylethane (compound 4-2)

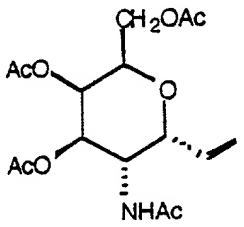


To a solution of the compound (0.14g, 0.29mmol) obtained from the above mentioned Example 8 in dichloromethane (2ml) was slowly added a solution of 3-chloroperoxybenzoic acid in dichloromethane (1.0ml) at -78°C. After stirring for 30min, diethyl ether (10ml) and 10% NaOH (1ml) was added to the reaction mixture and the mixture was stirred for 15min. The organic layer was separated and washed with water and brine. After drying ($MgSO_4$), the solvent was removed under reduced pressure. 0.15g (99%) of the

objective compound was obtained as a colorless oil.

In the paragraph at Page 53, line 20 to Page 54, line 5:

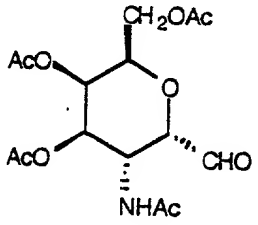
The preparation of 3-(2-acetylamino-[3,4-tri]3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-1-vinylene (compound 4-3)



A mixture of the compound (0.14g), 0.29mmol) obtained from the above mentioned Example 9 and diisopropylethylamine (0.09ml) in toluene (2ml) was refluxed for 18h. After the reaction mixture was cooled to room temperature, the mixture was extracted with ethyl acetate and washed with water and brine. After drying ($MgSO_4$), the solvent was removed under reduced pressure. The resulting residue was purified by silicagel column chromatography (BW-200, AcOEt). 0.07g (70%) of the oily objective compound was obtained as a colorless oil.

In the paragraph at Page 54, lines 11-18:

The preparation of 3-(2-acetylamino-[3,4-tri]3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-1-carbaldehyde (compound 4-4)

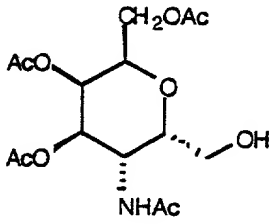


To a mixture of the compound (0.07g, 0.20mmol) obtained from the above mentioned Example 10, in tetrahydrofran (2ml) and water was added $NaIO_4$ (0.16g, 0.78mmol) and 4% OsO_4 solution (0.01ml). After the mixture was stirred for 4h, the reaction mixture was extracted with ethyl acetate and washed with water and brine. After drying ($MgSO_4$), the solvent was removed under reduced pressure. 0.705g (69.6%) of the objective aldehyde compound was obtained as a colorless oil.

In the paragraph at Page 54, line 24 to Page 55, line 6:

The preparation of 3-(2-acetylamino-[3,4-tri]3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-1-methanol

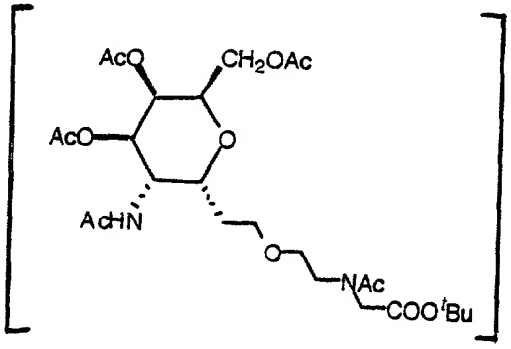
(compound 4-5)

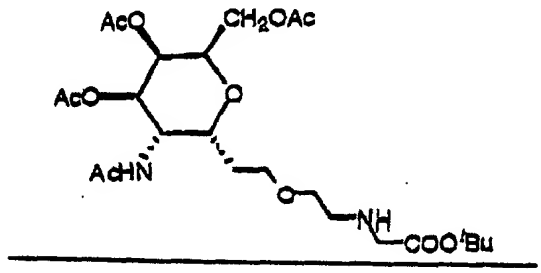


A mixture of the compound (0.77g, 1.85mmol) obtained from the above mentioned Example 11 and sodium borohydride (0.1g, 2.78mmol) in methanol (10ml) was stirred for 10min at 0°C. The reaction mixture was poured into satd. NH_4Cl and the mixture was extracted with dichloromethane, the organic layer was washed with water and brine. After drying ($MgSO_4$), the solvent was removed under reduced pressure. The resulting residue was purified by silicagel column chromatography (BW-200, AcOEt). 0.25g (36%) of the objective alcohol compound was obtained as a colorless oil.

In the paragraph at Page 58, lines 7-13:

The preparation of t-butyl 2-[(2-{2-[2-acetylamino-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl]ethoxy}ethyl)amino]acetate (compound 5-5a)





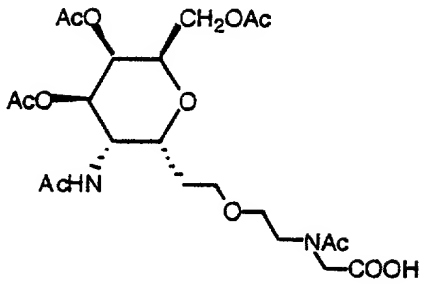
The compound (0.21g, 0.34mmol) obtained from the above mentioned Example 16 disolved in methanol (10ml), acetic acid (0.5ml) and 10% Pd-C(20mg) were added to the solution. The reaction mixture was stirred for 3h under an atmosphere of H_2, then the suspension was filtered through celite and the filtrate was concentrated. 0.18g (99%) of the objective compound was obtained as a colorless oil.

In the paragraph at Page 59, lines 11-16:

The preparation of 2-[N-(2-{2-[2-acetylamino-3,4,6-tri-O-

acetyl-2-deoxy-α-D-

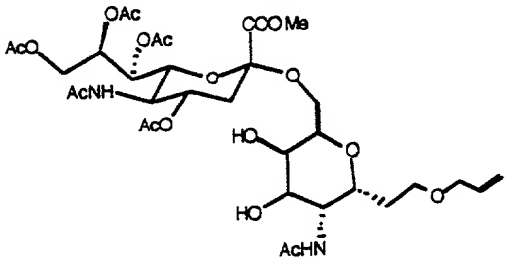
galactopyranosyl]ethoxy}ethyl)[benzylamino]acetylamino]acetic acid (compound 5-7a)



A mixture of the compound (0.15g, 0.26mmol) obtained from the above mentioned Example 18 and trifluoroacetic acid (0.4ml) in dichloromethane (2ml) was added to the mixture and stirred for 3h. The reaction mixture was concentrated and 0.13g (66.8%) of the objective compound was obtained as a colorless oil.

In the paragraph at Page 60, lines 2-11:

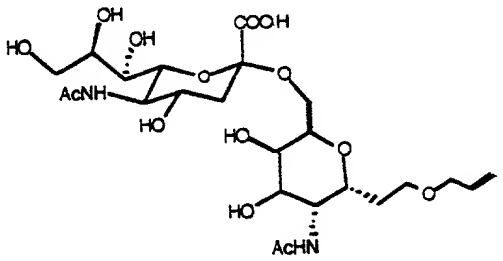
The preparation of O-(methyl 5-acetylamino-[4,8,9-tetra]4,7,8,9-tetra-O-acetyl-3,5-dideoxy-β-D-glycero-D-galacto-2-[nuno pyranosynate]nonulopyranoside)-2→6)-2-(2-acetylamino-3,4-di-O-acetyl-2-deoxy-α-D-galactopyranosyl)-1-(prop-2-enyloxy)ethane (compound 6-2a)



A mixture of the alcohol compound (173mg, 0.66mmol) obtained from the above mentioned Example 14 and MS4A (380g) in tetrahydrufuran (10ml) was added di-tert-butylpyridine (0.29ml) and AgOTf (337mg) and the mixture was stirred for 30min. After cooling to -78°C, a solution of the sialyl chloride (670mg, 0.66mmol) in tetrahydrofurane (8ml) was added dropwise to the mixture and the mixture was stirred for 28h. The suspension was filtered through Celite and the filtrate was removed under reduced pressure. The resulting residue was purified by silicagel column chromatography (CHCl3:MeOH=10:1). 81mg (18%) of the objective compound was obtained as a colorless oil.

In the paragraph at Page 60, line 19 to Page 61, line 5:

The preparation of O-(methyl 5-acetylamino-3,5-dideoxy-β-D-glycero-D-galacto-2-[nuno pyranosinate]nonulopyranoside)-(2→6)-2-(2-acetylamino-2-deoxy-α-D-galactopyranosyl)-1-prop-2-enyloxy)ethane (compound 6-3a)

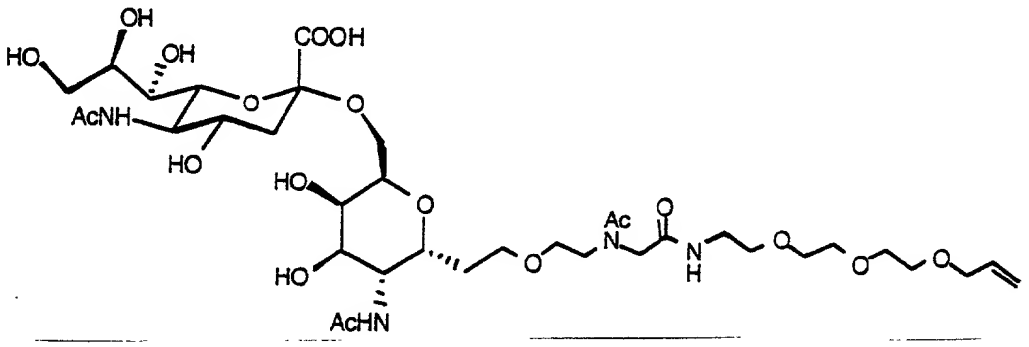


A mixture of the compound (21mg, 0.027mmol) obtained from the above mentioned Example 20 and 2% K_2CO_3 (3ml) in methanol (9ml) was stirred for 20h. The reaction mixture was neutralized by 1% HCl, then the reaction mixture was concentrated under reduced pressure. The resulting residue was purified by silicagel column

chromatography (PR-18, H2O:AcOH=100:1). 13mg (81%) of the objective compound was obtained as a colorless oil.

In the paragraph at Page 61, lines 13-18:

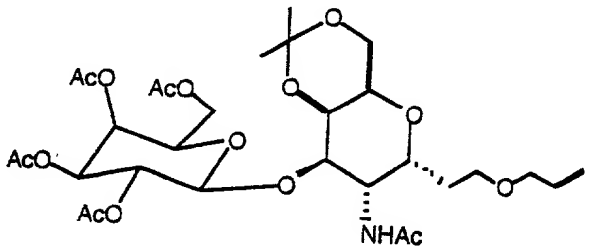
The preparation of O-(methyl 5-acetylamino-3,5-dideoxy-β-D-glycero-D-galacto-2-[nonuropysanosinate]nonulopyranoside)-(2→6)-[N-(2-{2-[2-acetylamino-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl]ethoxy}ethyl)acetylamino]-N-(2-{2-[2-(2-[oxyoethoxy]propenyloxyethoxy)ethoxy]ethoxy}ethyl)acetamide (6-3b)



To use of the compound obtained from the following mentioned Example 32, the objective compound was obtained according to the method described in Example 20-21.

In the paragraph at Page 62, line 19, to Page 63, line 6:

The preparation of the following compound.

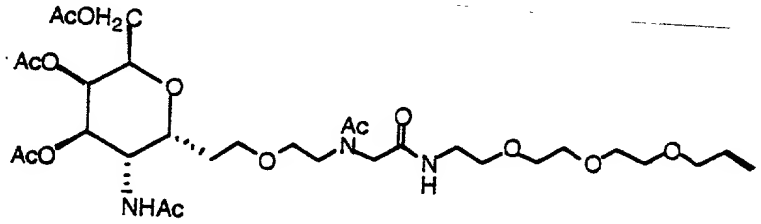


A mixture of the compound (100mg, 0.41mmol) obtained from the

above mentioned Example 23 and MS4A (380g) in dichloromethane (10ml) was added di tert-butylpyridine (0.12ml) and AgOTf (0.14g) and the mixture was stirred for 30min. After cooling to -78°C, a solution of the galactose derivatives (0.22g 0.41mmol) in dichloromethane was added dropwise to the mixture. After the reaction was completed, the solvent was removed under reduced pressure. The resulting residue was purified by silicagel column chromatography (BW-200, AcOEt). 0.10g (64.4%) of the objective compound was obtained as a colorless oil.

In the paragraph at Page 67, lines 1-11:

The preparation of 2-(2-Acetylamino-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-1-(2-{N-[(N-{2-[2-([2-prop 2-enyloxyethoxy]2-propenyloxyethoxy)ethoxy]ethyl}carbamoyl)methyl] acetylamino}ethoxy)ethane (compound 8-4)

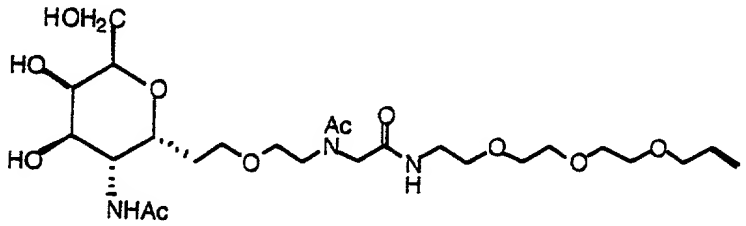


To a solution of carboxylic acid (23mg, 44.4μmol) obtained from above mentioned Example 19 and amine (17mg, 88.8μmol) in acetonitrile (1ml) was added diisopropylethylamine (9μl, 48.8μmol), O-(benzotriazol-1-yl)N,N,N′,N′-tetramethylhydroniumtetrafluoroborate (TBTU) (16mg, 48.8μmol). After the mixture was stirred for 4h, the mixture was poured into brine and extracted with chloroform, the organic layer was washed with 10% HCl and satd. $NaHCO_3$. After drying (Na_2SO_4), the solvent

was removed under reduced pressure and the resulting residue was purified by silicagel chromatography (AcOEt:MeOH=8:1). 20mg (65%) of the objective compound was obtained as a colorless oil.

In the paragraph at Page 67, line 19 to Page 68, line 1:

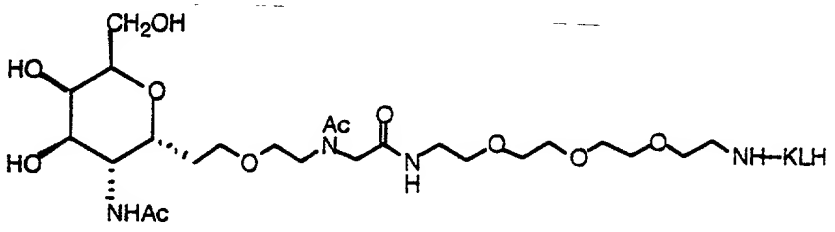
The preparation of 2-[N-(2-{2-[2-Acetylamino-2-deoxy-α-D-galactopyranosyl]ethoxy}ethyl)acetylamino]-N-}2-]2-([2-prop-2-oxyethoxy]2-propenyloxyethoxy)ethoxy]ethyl}acetamide (compound 8-6)



A mixture of the acetate compound (19.5mg, 29.0μmol) obtained from the above mentioned Example 31 and sodium methoxide (3mg, 58.0μmol) in methanol (1ml) was stirred for 1.5h at 0°C. The reaction mixture was neutralized by IR-120, filtered and the filtrate was removed under reduced pressure. 15.7mg (99%) of the objective compound was obtained as a colorless oil.

In the paragraph at Page 68, line 20 to Page 69, line 3:

The preparation of the following compound.



A solution of the compound obtained from the above mentioned Example 32 in methanol and dichlorometane was ozonized at -78°C.

The reaction mixture was treated with dimethylsulfide and concentrated to obtain the aldehyde. To the mixture of this aldehyde and KLH in phosphate buffer was added sodium cyanoborohydride and stirred for 30h. After purified by dialysis using PBS(-), the objective glycoprotein antigen was obtained.

Clean copies of the above-amended paragraphs are attached hereto.

IN THE CLAIMS

Please cancel claims 5-7 and 9-16 in their entirety and without prejudice.

Please enter the following new claims:

--17. (New) The non-mucin type synthetic compound or carrier conjugated compound thereof of claim 1, wherein A is sialic acid and/or its derivatives and B is OH.

18. (New) The non-mucin type synthetic compound or carrier conjugated compound thereof of claim 2, wherein A is sialic acid and/or its derivatives and B is OH.

19. (New) The non-mucin type synthetic compound or carrier conjugated compound thereof of claim 3, wherein A is sialic acid and/or its derivatives and B is OH.

20. (New) The non-mucin type synthetic compound or carrier conjugated compound thereof of claim 4, wherein A is sialic acid

and/or its derivatives and B is OH.

21. (New) The non-mucin type synthetic compound or carrier conjugated compound thereof of claim 1, wherein A is OH and B is galactose and/or its derivatives.

22. (New) The non-mucin type synthetic compound or carrier conjugated compound thereof of claim 2, wherein A is OH and B is galactose and/or its derivatives.

23. (New) The non-mucin type synthetic compound or carrier conjugated compound thereof of claim 3, wherein A is OH and B is galactose and/or its derivatives

24. (New) The non-mucin type synthetic compound or carrier conjugated compound thereof of claim 4, wherein A is OH and B is galactose and/or its derivatives

25. (New) The non-mucin type synthetic compound or carrier conjugated compound thereof of claim 1, wherein both A and B are OH.

26. (New) The non-mucin type synthetic compound or carrier conjugated compound thereof of claim 2, wherein both A and B are OH.

27. (New) The non-mucin type synthetic compound or carrier conjugated compound thereof of claim 3, wherein both A and B are OH.

28. (New) The non-mucin type synthetic compound or carrier conjugated compound thereof of claim 4, wherein both A and B are OH.

29. (New) Immunotherapy using the non-mucin type synthetic

compound or carrier conjugated compound thereof of claim 1.

30. (New) Immunotherapy using the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 2.

31. (New) Immunotherapy using the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 3.

32. (new) Immunotherapy using the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 4.

33. (New) Monoclonal antibodies prepared using the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 1.

34. (New) Monoclonal antibodies prepared using the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 2.

35. (New) Monoclonal antibodies prepared using the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 3.

36. (New) Monoclonal antibodies prepared using the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 4.

37. (New) Antitumor agents containing the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 1 as an active ingredient.

38. (New) Antitumor agents containing the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 2 as an active ingredient.

39. (New) Antitumor agents containing the non-mucin type

synthetic compound or carrier conjugated compound thereof of claim 3 as an active ingredient.

40. (New) Antitumor agents containing the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 4 as an active ingredient.

41. (New) Tumor immunostimulant containing the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 1 as an active ingredient.

42. (New) Tumor immunostimulant containing the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 2 as an active ingredient.

43. (New) Tumor immunostimulant containing the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 3 as an active ingredient.

44. (New) Tumor immunostimulant containing the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 4 as an active ingredient.

45. (New) Anti human immunodeficiency virus (HIV) agents containing the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 1 as an active ingredient.

46. (New) Anti human immunodeficiency virus (HIV) agents containing the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 2 as an active ingredient.

47. (New) Anti human immunodeficiency virus (HIV) agents containing the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 3 as an active ingredient

48. (New) Anti human immunodeficiency virus (HIV) agents containing the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 4 as an active ingredient.

49. (New) An immunostimulant for human immunodeficiency virus (HIV) containing the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 1 as an active ingredient.

50. (New) An immunostimulant for human immunodeficiency virus (HIV) containing the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 2 as an active ingredient.

51. (New) An immunostimulant for human immunodeficiency virus (HIV) containing the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 3 as an active ingredient.

52. (New) An immunostimulant for human immunodeficiency virus (HIV) containing the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 4 as an active ingredient.

53. (New) A therapeutic method for tumor treatment using the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 1 as an active ingredient.

54. (New) A therapeutic method for tumor treatment using the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 2 as an active ingredient.

55. (New) A therapeutic method for tumor treatment using

the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 3 as an active ingredient.

56. (New) A therapeutic method for tumor treatment using the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 4 as an active ingredient.

57. (New) A therapeutic method for treatment of HIV using the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 1 as an active ingredient.

58. (New) A therapeutic method for treatment of HIV using the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 2 as an active ingredient.

59. (New) A therapeutic method for treatment of HIV using the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 3 as an active ingredient.

60. (New) A therapeutic method for treatment of HIV using the non-mucin type synthetic compound or carrier conjugated compound thereof of claim 4 as an active ingredient.--

REMARKS

Entry of the foregoing amendments prior to examination of this application is respectfully requested in view of the following comments.

Claims 1-16 are currently pending in this application. Claim 5-7 and 9-16 have been cancelled and new claims 17-60 added. Accordingly, claims 1-4, 8 and 17-60 are herewith presented for examination.

The specification has been amended to correct typographical errors which occurred in typing the English language translation from the original Japanese. In particular the written compound names have been amended so as to conform to the compound structure depicted in the particular paragraph.

No new matter has been added.

Claims 5-7 have been cancelled to eliminate their multiple dependency and the extra fee therefor and new claims 17-28 correspond to claims 5-7 rewritten in single dependent form.

Claims 9-16 have been cancelled to eliminate their improper multiple dependencies and new claims 29-60 correspond to claims 9-16 rewritten in single dependent form dependent from claims 1-4.

Thus, the amendments herein have been made to correct typographical errors, improper claim forms and to reduce the filing fees therefor.

No new matter has been added by these amendments and applicants respectfully submit that this application is in condition for allowance and an early notice to that effect is earnestly solicited.

Respectfully submitted,

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